StagePresser™ User's Manual

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Culturing Cells in a Mechanically Active Environment™
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USING THE STAGEPRESSER™

The StagePresser™ is designed to compress a single tissue sample or cells in a gel while viewing the cellular activity under a microscope. The StagePresser™ uses a piston adhered to a rubber membrane to apply force to a sample in culture. The piston is moved upward by positive air pressure applied to the silicone membrane. A Flexcell® FX-5000™ Compression System controls the compression frequency, amplitude, waveform, and cycles (or time period).

STAGEPRESSER™ ASSEMBLY AND USE

Initial assembly of the StagePresser™ involves the following parts:

1. Main body of the StagePresser™
2. Black rubber sealing disk
3. StagePresser™ silicone membrane/ piston/ sample holder assembly
4. O-ring
5. Stationary top
6. Adjustable height top with O-ring, glass window, and clamping ring.

The StagePresser™ should be assembled when the device arrives and should remain assembled during use. Normal use of the device requires only the removal and adjustment of the adjustable height top and the sample holder inside of the chamber.

The tissue or culture sample is placed into the small foam sample holder at the center of the silicone membrane/ piston/ sample holder assembly inside of the StagePresser™. The thickness of the gel or tissue sample should be no less than 1000 µm to ensure compressibility. The thickness of the compressed foam is 350 µm, therefore thinner samples will not be compressed.

The sample diameter should be equal to or less than the inner diameter of the foam sample holder in the chamber, which is 0.200” (5 mm). If you wish to use a larger sample, you can remove the sample holder and place your sample within the larger ring that normally contains the sample holder. This will allow a sample diameter of up to 0.525” (13 mm).

Once the sample is placed within the chamber, the adjustable height top is screwed into the body of the StagePresser™, then turned clockwise until the sample just begins to contact the bottom of the glass. If you wish to determine the exact number of revolutions required, turn the adjustable top until its surface is exactly flush with the top surface of the StagePresser™ body, then see the following instructions and equations below.

NOTE: *If your sample height is greater than 0.150” (3.81 mm), turning the adjustable top to the position where its surface is flush with the StagePresser™ body will begin to compress your sample. In this case, you will want to disassemble the StagePresser™ body by removing the four screws around the periphery, then adjust the top (according to the results obtained from the following equations) after the body is removed from the base. Once the top is adjusted, reassemble the base and body with the sample already in place in the sample holder. As the body is assembled, be careful to insure that the membrane/piston assembly is accurately centered over the chamber in the base of the StagePresser™, and that the O-ring is centered over the O-ring groove around the periphery of the base.*

Use the ‘*x*’ value in the following equation(s) to determine the turning direction and how many revolutions will be

required to begin contacting the top of your sample.

If your sample height is measured in millimeters:

$$x = (3.81 - h_s) / 0.39$$

where *x* is the number of 180 degree turns required of the adjustable platen [(+) number = clockwise turns; (-) number = counterclockwise turns] and h_s is the sample height in millimeters.

If your sample height is measured in inches:

$$x = (0.150 - h_s) / 0.015$$

where *x* is the number of 180 degree turns required of the adjustable platen [(+) number = clockwise turns; (-) number = counterclockwise turns] and h_s is the sample height in inches.

Use the 8 medium addition holes around the periphery of the adjustable top in conjunction with the four screw holes around the periphery of the StagePresser™ body to monitor the number of revolutions or partial revolutions of the adjustable top.

Add medium to the cells or tissue as needed (3-5 mls) through the holes around the periphery of the adjustable top.

The base of the assembly should fit onto your microscope base for proper viewing. A larger base is also included should you wish to screw the device onto your microscope. Simply remove the four screws in the bottom of the small base and reassemble the device with the larger base.

To view your tissue sample or gel under a microscope, a lighting source from above will be required. Direct the source to the sample and adjust as necessary to illuminate the sample for viewing with your microscope. An upright or standard microscope is required for viewing the 3D sample.

DEVICE MAINTENANCE

Once an experiment is complete, remove the medium and wash the device with deionized water. If the device is not washed after each experiment, the aluminum will eventually oxidize and lose its aesthetic quality.

The adjustable height top has a thin layer of Teflon® tape around the threads on the side. This tape keeps the top from vertical movement during compression as a result of unwanted space between threads. If this tape should wear off such that the adjustable height top is no longer vertically stable within the StagePresser™, apply another thin layer of Teflon® tape to the threads with the small roll supplied in the accessories kit.

USING THE STAGEPRESSER™ WITH THE FX-5000™ COMPRESSION SYSTEM

Connect the front quick disconnect of the StagePresser™ to the FX-5000™ as shown below in Figure 1.

Connect the white PVC pipe volume (included with the system) inline with the larger clear tubing on the FX-5000™ tubing connector. Cut the tubing on the adapter at its midpoint. When assigning a regimen for the StagePresser™, simply choose the platform ***StagePresser*** when downloading the regimen. This will specify the proper conversion data for the computer as it regulates the specific forces you have requested. If you do not have ***StagePresser*** as an option under the platform drop-down menu, please contact Flexcell.

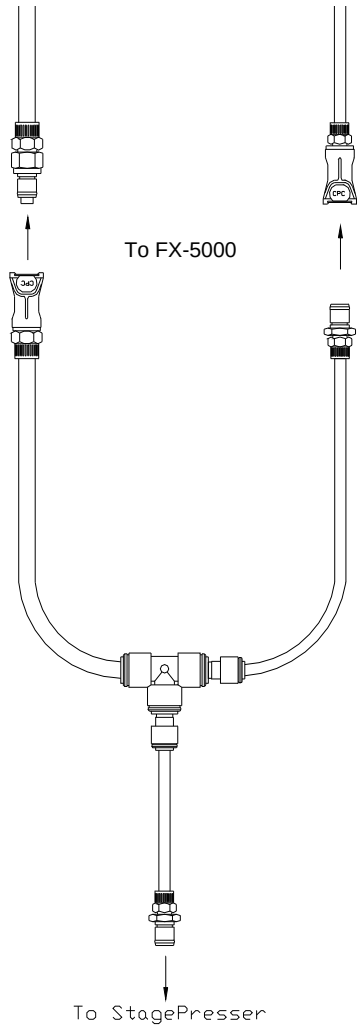

Figure 1. StagePresser™ to FX-5000™ Compression Unit tubing adapter

NOTE: *In programming regimens for the StagePresser™ with the FX-5000™ FlexSoft® program, force (in pounds) is used instead of minimum and maximum % elongation. Therefore, keep in mind that you are specifying the minimum and maximum force in pounds when programming regimens, not minimum and maximum % elongation.*

APPENDICES

Appendix 1: StagePresser™ Compression Conversion Chart

APPENDIX 1: STAGEPRESSER™ COMPRESSION CONVERSION CHART

Press (kPa)	Force (lb)	Press (kPa)	Force (lb)	Press (kPa)	Force (lb)	Press (kPa)	Force (lb)
0.00	0.0	27.41	5.4	54.81	10.8	82.22	16.2
0.51	0.1	27.91	5.5	55.32	10.9	82.72	16.3
1.02	0.2	28.42	5.6	55.83	11.0	83.23	16.4
1.52	0.3	28.93	5.7	56.33	11.1	83.74	16.5
2.03	0.4	29.44	5.8	56.84	11.2	84.25	16.6
2.54	0.5	29.94	5.9	57.35	11.3	84.75	16.7
3.05	0.6	30.45	6.0	57.86	11.4	85.26	16.8
3.55	0.7	30.96	6.1	58.36	11.5	85.77	16.9
4.06	0.8	31.47	6.2	58.87	11.6	86.28	17.0
4.57	0.9	31.97	6.3	59.38	11.7	86.78	17.1
5.08	1.0	32.48	6.4	59.89	11.8	87.29	17.2
5.58	1.1	32.99	6.5	60.39	11.9	87.80	17.3
6.09	1.2	33.50	6.6	60.90	12.0	88.31	17.4
6.60	1.3	34.00	6.7	61.41	12.1	88.81	17.5
7.11	1.4	34.51	6.8	61.92	12.2	89.32	17.6
7.61	1.5	35.02	6.9	62.42	12.3	89.83	17.7
8.12	1.6	35.53	7.0	62.93	12.4	90.34	17.8
8.63	1.7	36.03	7.1	63.44	12.5	90.84	17.9
9.14	1.8	36.54	7.2	63.95	12.6		
9.64	1.9	37.05	7.3	64.45	12.7		
10.15	2.0	37.56	7.4	64.96	12.8		
10.66	2.1	38.06	7.5	65.47	12.9		
11.17	2.2	38.57	7.6	65.98	13.0		
11.67	2.3	39.08	7.7	66.48	13.1		
12.18	2.4	39.59	7.8	66.99	13.2		
12.69	2.5	40.09	7.9	67.50	13.3		
13.20	2.6	40.60	8.0	68.01	13.4		
13.70	2.7	41.11	8.1	68.51	13.5		
14.21	2.8	41.62	8.2	69.02	13.6		
14.72	2.9	42.12	8.3	69.53	13.7		
15.23	3.0	42.63	8.4	70.04	13.8		
15.73	3.1	43.14	8.5	70.54	13.9		
16.24	3.2	43.65	8.6	71.05	14.0		
16.75	3.3	44.15	8.7	71.56	14.1		
17.26	3.4	44.66	8.8	72.07	14.2		
17.76	3.5	45.17	8.9	72.57	14.3		
18.27	3.6	45.68	9.0	73.08	14.4		
18.78	3.7	46.18	9.1	73.59	14.5		
19.29	3.8	46.69	9.2	74.10	14.6		
19.79	3.9	47.20	9.3	74.60	14.7		
20.30	4.0	47.71	9.4	75.11	14.8		
20.81	4.1	48.21	9.5	75.62	14.9		
21.32	4.2	48.72	9.6	76.13	15.0		
21.82	4.3	49.23	9.7	76.63	15.1		
22.33	4.4	49.74	9.8	77.14	15.2		
22.84	4.5	50.24	9.9	77.65	15.3		
23.35	4.6	50.75	10.0	78.16	15.4		
23.85	4.7	51.26	10.1	78.66	15.5		
24.36	4.8	51.77	10.2	79.17	15.6		
24.87	4.9	52.27	10.3	79.68	15.7		
25.38	5.0	52.78	10.4	80.19	15.8		
25.88	5.1	53.29	10.5	80.69	15.9		
26.39	5.2	53.80	10.6	81.20	16.0		
26.90	5.3	54.30	10.7	81.71	16.1		